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Forest microhabitats differentially influence seedling phenology of two co-existing Mediterranean oak species

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Abstract

Question: Climate change and drought episodes are predicted to alter future species distribution by affecting the phenology of tree species. In Mediterranean ecosystems, the ability of seedlings to cope with summer drought can be influenced by microhabitat conditions. We ask how forest microhabitats impact seasonal microclimatic conditions and phenological seedling strategies in two co-occurring Mediterranean *Quercus* species with different leaf habits.

Location: *Pinus halepensis* woodlands, Provence, SE France.

Methods: Ninety sets of three acorns of *Quercus ilex* (holm oak, evergreen) or *Quercus pubescens* (downy oak, winter deciduous) were sown under various *P. halepensis* canopy cover conditions. Canopy cover and litter depth were measured at each sowing point, and soil moisture and temperature were measured twice-monthly. Seedling phenology and aerial development were monitored during the second growing season under different microhabitats.

Results: During rainy periods, soil moisture was higher under open canopy cover and thinner litter layers, whereas during summer drought soil moisture was higher under dense canopy and thicker litter layers. *Q. pubescens* seedlings presented higher growth activity than *Q. ilex* before summer drought, whereas the pattern reversed during autumnal growth. Dense canopy cover strongly delayed budburst timing and decreased foliar development in *Q. pubescens*, which was more affected than *Q. ilex*. Depending on species phenology, litter thickness can influence the height growth of flushes occurring during early summer.

Conclusions: *Q. pubescens* has a phenological strategy that allows it to develop a maximal foliar area before summer drought, which is a typical growth strategy of Mediterranean winter deciduous species. In contrast, the later phenological activity of the evergreen *Q. ilex* enables it to cope with the dry period and capitalize on autumnal precipitation events. A dense cover acts as shelter in the dry season but can reduce soil moisture during wetter seasons. In contrast with *Q. ilex*, the phenological response of *Q. pubescens* appeared strongly dependent on canopy cover, which would suggest that *Q. ilex* has a larger regeneration niche in Aleppo pine woodlands. In a context of global change, this "microhabitat effect" on species-specific phenological strategy underlines the need to consider the effects of local environmental conditions on regenerating species.

Key-words: Canopy; Litter; Soil moisture; *Quercus ilex*; *Quercus pubescens*; Phenology; Survival

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Introduction

Climate models predict Mediterranean-wide warming and drying, with extreme drought episodes and a significant decrease in soil wetness (Gibelin & Deque 2003, Anon 2007). These drought events may have stronger impacts in water-limited areas, where the intensification of drastic conditions for plant development increases the vulnerability of Mediterranean ecosystems (Knapp et al. 2002, Vicente-Serrano et al. 2010).

Summer drought is recognized as a major bottleneck in Mediterranean tree dynamics, especially during periods critical to seedling establishment (Gomez-Aparicio et al. 2005a). Although most studies focus on survival and growth, other more recent studies have underlined the importance of considering phenological development in response to drought (Montserrat-Marti et al. 2009, Morin et al. 2010). Summer drought can advance budburst in the following season by an increase in abscisic acid under drought (Rinne et al. 1994, Arora et al. 2003), which arrests plant growth and induces earlier bud formation (Horvath et al. 2003). Because leaf expansion is developmental behaviour particularly vulnerable to water deficit (Van Volkenburgh 1999), forest tree species could show an adaptation to arid environments through rapid leaf growth before the driest period (Radoglou 1996, Fotelli et al. 2000). Reducing the duration of leaf development may help avoid the negative effects of severe drought and limit water use through transpiration (Misson et al. 2011). If favourable climatic conditions prevail during autumn, then saplings of Mediterranean tree species show a second period of vegetative growth (Gratani et al. 1992).

Climate change has regional and global dimensions, but community structure and species recruitment are largely shaped at a smaller scale by microhabitats that can modulate the negative impacts of drought on seedling development. Litter thickness or canopy structure can modify local irradiance, soil temperature and soil moisture, thus influencing survival (Callaway 1995, Valladares et al. 2005, Sayer 2006, Rodriguez-Calcerrada et al. 2010) and seedling phenology (Sanz-Pérez et al. 2010). For example, as the viscosity of water is directly proportional to water temperature (Kramer 1934), cold soil will reduce water transport (Carlson and Miller 1991) and thus retard cell elongation and delay budburst. Studying the effects of irradiance on phenology, Sanz-Pérez et al. (2010) found that shade reduced plant carbohydrates, and assumed that the delayed bud development delay under dense canopy cover could result from carbon shortage, as buds require carbon for both development and dormancy release (Kozlowski 1992; Marquat et al. 1999). This has given rise to the hypothesis that the adaptive early and rapid leaf deployment of understory saplings before summer drought may be constrained by the effects of a dense canopy cover by altering determinant factors of phenology (irradiance, temperature and soil moisture;

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Morin et al. 2010, Sanz-Pérez & Castro-Díez 2010). Furthermore, in semi-arid ecosystems, the effects of canopy cover may shift from negative effects during rainy spring periods (rainfall interception, irradiance shortage) to positive effects during dry summer periods (conserving soil moisture, limiting photoinhibition), thus differentially affecting the summer mortality and autumn phenology of seedlings under dense or open canopies.

In the French Mediterranean area, the current *Pinus halepensis* Mill. forests are expected to be naturally replaced by successional oak species (Barbero et al. 1990, Lookingbill & Zavala 2000). Two late-successional oak species with contrasting leaf habits coexist in this region: the evergreen *Quercus ilex* L. and the winter deciduous *Quercus pubescens* Willd. The issue is to identify the conditions under which either of these two species will predominantly replace *P. halepensis* and whether forest microhabitats play a role in the process. *Q. pubescens* shows earlier and more rapid development of shoots and leaves (Du Merle & Mazet, 1983) than *Q. ilex*, whereas *Quercus faginea* Lam (another Mediterranean winter deciduous species) shows lower phenological activity than *Q.* ilex during summer. This phenological behaviour indicates a tendency to 'avoid' rather than 'tolerate' summer drought for deciduous species (Montserrat-Marti et al. 2009). Thus, under dense canopy cover, modification of micro-conditions may differentially affect the phenological strategies of *Q. ilex* and *Q. pubescens* by delaying spring budburst or compromising autumnal growth.

The main goal of the present study was to assess the effect of *P. halepensis* canopy cover and litter thickness on the phenology of the two coexisting Mediterranean oak-species seedlings in response to summer drought conditions. The research reported here tackles two key questions: (1) Does the canopy cover and litter thickness of *P. halepensis* induce a seasonal shift from positive to negative effects on microhabitat quality? (2) Are there differential canopy and litter effects on the phenological strategies of *Q. ilex* and *Q. pubescens*? To answer these questions, we monitored soil micro-conditions and the seedling growth and phenology of both species over one year under different canopy cover and litter depth conditions in a *P. halepensis* Mediterranean forest.

Materials and methods

Study site and experimental design

The study site (mean altitude 130 m) was located in South-East France approximately 25 km north-west of Marseille in a 400-hectare area covered mainly with 50-year old *P. halepensis* woodlands (43°27'N, 5°2'E). Climate is meso-Mediterranean with a summer drought period. Mean annual temperature is 14.5°C. Mean annual rainfall over the 1961-1996 period, based on data from the nearby weather station (Istres), was 406 mm. Soils are

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calcareous with a loamy-sandy texture, a low stone load, and a depth varying between 20 and 40 cm. This area has undergone a long history of fire and land use (mainly croplands), but is relatively well preserved, with no record of fire for at least 50 years. Site vegetation is composed of a monospecific dominant *P. halepensis* tree-layer but with a variable cover, a weakly developed subcanopy layer of *Q. ilex*, a highly spatially heterogeneous shrub layer mainly dominated by *Quercus coccifera* L., *Quercus ilex* L., *Rosmarinus officinalis* L., *Phyllerea angustifolia* L., and a sparse grass layer. *Q. pubescens* individuals are occasionally found in and around the study site. This scarce occurrence is due more to a long history of land use than to ecological limiting conditions. Human activities probably favoured the extension of *Q. ilex* at the expense of *Q. pubescens*, in part to benefit the higher resprout capacity of the *Q. ilex* (Barbero et al. 1990).

The experiment started in October 2007 and follow-up data were collected over 2008 and 2009. Acorns of *Q. ilex* and *Q. pubescens* were collected from several trees at different sites located in two areas: (i) near La Roque d'Anthéron (43°42'N, 5°21'E), altitude = 186 m, mean annual precipitation = 637 mm, mean annual temperature = 12.7°C, distance from study site = 36 km; (ii) near Grans (43°36'N, 5°3'E), altitude = 100 m, mean annual precipitation = 601 mm, mean annual temperature = 13.7°C, distance from study site = 15 km. Non-viable acorns were eliminated by the floating method and visual screening (acorn fresh weight was *Q. pubescens* = 4.1 g.±0.8 and *Q. ilex* = 2.9 g.±0.4). We sowed a total of 90 sets (half *Q. pubescens* and half *Q. ilex*) of three acorns of each species (sowing points). The sowing points of both species were selected by visual estimation to cover the widest possible range of canopy cover. Sowing points of the two species were placed close together (< 2 m) to ensure comparable microhabitat conditions. The sowing points were covered by 3 cm soil, then a wire mesh (10 cm × 10 cm, 0.6 cm mesh size – the wire mesh was previously bathed 24 h in an acidic solution to promote corrosion) was placed flat on the soil to exclude seed predators, then covered in another 2 cm of soil. Litter was carefully removed and replaced before and after sowing to maintain the same litter structure. No predation was observed on sown acorns.

Characterization of microhabitats

We characterized the aboveground (light availability and litter depth) and belowground (soil moisture and temperature) environment of each of the 90 experimental sowing plots. Light availability was quantified by hemispherical photography. Photographs were taken in April 2009 at seedling level just above the uppermost leaves of every sampled sapling, using a digital plant canopy imager (CI-110, CID Inc., USA). All photographs were taken after sunset to ensure homogeneous illumination of the overstorey canopy and correct contrast

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between canopy and sky. The images were analyzed using GLA software® (Frazer et al. 1999). Light availability estimated by percentage canopy cover (CC) was used to create three classes of light conditions: Open canopy cover (45-80%, n=29), Medium canopy cover (20-45%, n=32) and Dense canopy cover (5-20%, n=29). Soil temperature and moisture (12 cm depth) were measured at each sowing point (and recorded as a mean of three readings per plot) using a time domain reflectometer probe (Wet sensor, Delta-T Devices). Measurements were performed every month during the growth period in the second year of the experiment (i.e. from March to October 2009), at the same time of day (2 pm ± 2 hours) each month to account for the high daily fluctuations of these variables (Verhoef et al. 2006, Andrade et al. 2010). Litter depth was estimated for each sampling point as the mean of three metric tape measurements. General estimations (n=228) were made in spring and autumn with non-continuous measurements, then two classes were used (thinner litter layer, litter depth < 6 cm, n=138, and thicker litter layer, litter depth > 6 cm, n=90) in data analysis. One isolated sampling was performed in dry summer in a subsample (n=41), with continuous measurements for higher precision.

Seedling Monitoring

Each seedling that emerged per sowing point in the spring of 2008 was individually tagged. Emergent seedlings were n=27, n=43, n=36 for *Q. pubescens* and n=34, n=49, n=39 for *Q. ilex* under Dense, Medium and Open cover, respectively, and n=53, n=53 for *Q. pubescens* and n=85, n=37 for *Q. ilex* in thinner and thicker litter layer, respectively. From April 2009, the seedlings were measured twice-monthly in spring and autumn (primary growth period) and monthly in summer (where most seedlings stopped growth). The apical bud of the 2009 leader shoot was monitored, and bud stage (according to Du Merle & Mazet 1983) was recorded for the first sampling date (3rd April 2009). For each seedling, we measured growth indicators linked to environmental factors (Chaar et al. 1997, Puntieri & Ghirardi 2010), i.e. number and length of the 2009 growth flushes and number and length of leaves when they developed enough to be measured. This dataset was used to analyze growth and phenological variables as described in Table 1. Foliar area of each seedling was computed using allometric relationships between foliar area and foliar length derived from two subsamples of n=60 individuals and n= 120 leaves per species: Area = exp(-0.985467 + 1.76573*ln(Length)), p-value < 0.0001, R^2=86% for *Q. pubescens*; and Area = exp(-0.796364 + 1.73784*ln(Length)), p-value < 0.0001, R^2=85% for *Q. ilex*.

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Data Analysis

For each comparison of each species between canopy cover classes at different dates, we used repeated measures ANOVA since the same individuals were monitored over time, including one between-subject canopy cover treatment with three levels (Open, Medium, Dense) and one within-subject factor (date). This analysis was performed for total height and total foliar area of seedlings from April 2009 to December 2009, and for soil moisture and temperature from April 2009 to October 2009. Significant differences between levels for each factor (3 for canopy cover, 11 for time of the year) were estimated by the Bonferroni post hoc test. Number of flushes and percentage of individuals growing per sowing point are not continuous variables and cannot be treated by parametric statistical analyses. These variables were compared between different canopy cover types throughout the year using Friedman's nonparametric test followed by Dunn's post hoc test to compare differences between the canopy cover classes. Non-parametric Kruskal-Wallis tests were also run on these two variables to analyze (i) date factor for each species and (ii) canopy cover factor in each species × date combination, followed by Dunn's post-hoc tests in the cases where differences were significant.

Species and canopy cover effects on (i) growth duration and (ii) growth parameters (height, foliar area, number of leaves, number of flushes, and proportion developed at a given date [see description of growth variables, Tab. 1 were analyzed using a Kruskal-Wallis test followed by a Dunn's post hoc test. Student tests were used to analyze species effects on height and foliage area. Because the assumption for normality was not fulfilled, Mann-Whitney non-parametric tests were used to analyze (i) species effects on bud development and on proportion of foliage developed at a given date, and (ii) litter rank effects on the height of the second flush of growth before the dry summer period. Pearson correlation coefficients (r_p) or Spearman rank correlation coefficients (r_s) were calculated between soil moisture and canopy cover or litter depth (depending on whether data were continuous). Concerning survival, we used the Wilcoxon test to test whether percentage survival for each species in each canopy cover differed significantly from 100%, then a Kruskall-Wallis test to analyze survival differences between species and between canopy covers. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, and Statgraphics Centurion XV (StatPoint, Inc., USA) software.

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Results

Environmental conditions: rainfall, soil moisture and soil temperature

Mean annual precipitation recorded at the nearest weather station was 779, 697 and 662 mm for 2008, 2009 and 2010 respectively, i.e. largely wetter than the mean of years 1961-1996 (406 mm). Main rainfall events during the studied period (April–October 2009) regularly occurred in spring (April–May), together with two intense but sporadic rainfall events in autumn (around 16th September and the 21st October) (Fig. 1). One isolated rainfall event was also observed in late spring (23 mm, around 7th June). No relevant precipitation events occurred between 10th June and 15th September (6.6 mm, Fig. 1).

Soil moisture was greater and more strongly affected by precipitation under Open than under Dense canopy cover during rainy periods (Fig. 1, two-way RM ANOVA: p <0.001). Indeed, during spring and autumn, soil moisture was higher under Open than Dense canopy, whereas during the strong summer drought in late July soils were slightly but significantly moister under Dense canopy cover (Fig. 1, for date = 24/07/09, p <0.001). This shift from positive to negative effects of canopy cover on soil moisture is illustrated in Figure 2. Canopy classes or soil moisture did not differ between species (Mann Whitney test, p>0.05).

During summer, mean soil temperatures ranged from 18°C to 30C°. Soil temperatures were higher under Open canopy cover than under Dense cover, with peak differences during the dry season (Fig. 3, two-way RM ANOVA: p <0.001). In Medium canopy cover, mean soil temperature was similar to that of the Open canopy cover during the rainy spring and to that of the Dense canopy cover during the summer dry period (Fig. 3, two-way RM ANOVA: p <0.001, Bonferroni post hoc test).

Survival

At the end of 2009, we found no significant mortality for either species (p >0.0001, Wilcoxon test) and no significant effects of species or canopy covers (Kruskal-Wallis, p >0.0001). Mean *Q. pubescens* survival was 95%, 97% and 90% in Open, Medium and Dense cover, respectively, and mean *Q. ilex* survival was 98%, 95% and 92% in Open, Medium and Dense cover, respectively.

Temporal development

Species-specific variability

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Growth duration was longer for *Q. ilex* than for *Q. pubescens*, independently of canopy cover (Kruskal-Wallis W=46.6, p <0.0001, Dunn's test). At the first sampling date (3rd April), buds were more developed for *Q. pubescens* than for *Q. ilex* seedlings, and by the second sampling date (23rd April), *Q. pubescens* presented more-developed foliage than *Q. ilex* (Table 2). *Q. pubescens* also presented a higher number of pre-summer flushes (Table 2) and higher foliage area before summer (24th June, t-test, t=4.510, df=226 p<0.0001) than *Q. ilex*. Both species practically stopped growing during the dry summer period (July and August, Fig. 4, 5). Before the summer drought, *Q. pubescens* seedlings showed a higher % final height, % final foliar area, % final flushes, and % final number of leaves than *Q. ilex*; (see Table 2), whereas the reverse pattern occurred in the autumn period.

Canopy effects

The earlier bud development observed for *Q. pubescens* compared to *Q. ilex* did not occur under Dense canopy cover (Kruskal-Wallis, p <0.0001, Dunn's test). Canopy covers also modified the between-species difference in the number of growth flushes. Under Open canopy cover, *Q. pubescens* produced three growth flushes (April, June, October) against only one growth flush (April) under Medium and Dense canopy cover (between-date comparisons in each canopy cover class for each species; Kruskal-Wallis, p <0.0001, Dunn's test). In contrast, *Q. ilex* showed two growth periods (April, October) in all micro-habitats (mean number of flushes and percentage of seedlings growing per sowing point; between-date comparisons in each canopy cover class for each species: Kruskal-Wallis, p <0.0001, Dunn's test). *Q. pubescens* showed greater leaf area under Open canopy cover than under Medium and Dense canopy cover from May, a higher number of flushes from 24th June, and a higher percentage of individuals growing in June and October (Fig. 4 D and 5 B, D). In contrast, Q. ilex showed greater growth activity (leaf area, number of flushes and percentage of individuals growing) under Open canopy than under Medium and Dense canopy from October only (Fig. 4 C and 5 A, C).

Seasonal effect of Litter depth

Litter depth was not significantly different between the three canopy classes (Kruskal-Wallis test, W=3.55, p >0.05). Note that the effects of canopy cover on plant performance (height and foliar area) were independent of litter depth (two-way ANOVA, canopy cover x litter depth, F_2= 0.1, p>0.05). The effect of litter depth on soil moisture was dependent on sampling period: during rainy periods, soil moisture was only weakly correlated with litter depth, while during dry summer soil moisture was more positively correlated with litter depth (Fig. 6). During dry summer, there was a higher increase of soil moisture under Open than Dense canopy

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cover (Fig. 6 A). The thickest litter layer had an effect on the height of *Q. pubescens* seedlings in the second (around June) flush of growth (Mann-Whitney U=20.50, $p<0.05$, mean=1.31 ± 0.25 n=10, mean=2.46 ± 0.39 n=10, for litter < 6cm and > 6cm, respectively) but not on *Q. ilex* seedlings (Mann-Whitney U=16.50, $p>0.05$). With regard to this specific effect, note that 67% (n=20) of the second flush produced by *Q. pubescens* occurred in June (when litter depth positively influenced soil moisture) against only 23% (n=14) for *Q. ilex*.

Discussion

Seasonal variability of the "canopy and litter effect" on soil moisture and soil temperature

We found that canopy covers influences soil micro-conditions regardless of litter depth. Compared to the Open canopy cover, the Dense canopy cover formed by adult trees has a negative effect on soil moisture during wetter seasons but acts as shelter and thus has a positive effect on soil moisture in dry seasons. Rainfall interception could be the main factor responsible for this difference in soil moisture between Dense and Open canopies during wetter seasons, whereas the balance between tree transpiration/evaporation could be the main factor during the dry summer season when rain events become scarce (Rey Benayas et al. 2005). Moreover, during summer, a higher soil temperature under Open Canopy than under Dense canopy could help increase the evaporative process and thus reduce soil moisture (Breshears et al. 1998). Our results suggest that the balance between positive and negative effects of neighbours (in this case adult trees) is dependent on seasonal climatic variability, in line with the theoretical predictions of Bertness and Callaway (1994) and Brooker and Callaghan (1998) asserting a shift from positive to negative effects with decreasing stress. This shift observed here supports the increasing importance of facilitation when abiotic stress (in this case drought) is intensified, as already reported in Mediterranean areas (Maestre et al. 2003, Lloret et al. 2005). However, these results occurred during two particularly wet years (2008-2009) inducing moderate water-stress for oak seedlings, as annual precipitation reached 779 and 697 mm, i.e. a 70 to 90% increase compared to the usual precipitation rate (406 mm). During more usual drier years, soil-water competition from adult trees may become more intensive and counter the benefits of the favourable transpiration/evaporation balance under Dense canopy (Bellot et al. 2004).

Interestingly, although litter cover showed the same shift from positive to negative effect depending on seasonal rainfall, an increase in drought intensity may enhance the relatively beneficial effect of litter depth on soil moisture. We compared the effect of litter depth on soil moisture between Open and Dense canopy during the drought period, and found a higher positive increment under the Open canopy (Fig. 6 A). The protective

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effect of litter layer is due to interception of radiation and reduced evaporation from the soil surface, thus explaining the higher soil moisture (Sayer 2006). However, such an effect may be largely dependent on the kind of rainfall events, with lower interception by pine canopy during major storm events (Bellot et al. 1999). Therefore, given the predicted seasonal changes in rainfall quantity and frequency in the Mediterranean area, it is particularly important to extend research on the interactions between the canopy and litter effects on soil moisture (IPCC 2007, Miranda et al. 2009, Vicente-Serrano et al. 2010).

Different phenological patterns between the two species in response to summer drought

Q. pubescens seedlings started bud and foliar development earlier than *Q. ilex* seedlings, as previously shown for winter deciduous and evergreen *Quercus* species (Sanz-Pérez & Castro-Díez 2010). This more rapid deployment of photosynthetic material in spring is an adaptation to arid environments that can confer an ecological advantage by benefitting from the favourable period conditions (Radoglou 1996, Fotelli et al. 2000). Both species practically stopped growing in summer, especially in July and August when summer drought is at its most intense, as an adaptive developmental strategy to avoid summer drought (Montserrat-Marti et al. 2009).

During the early summer period, *Q. pubescens* seedlings presented higher growth than *Q. ilex* and higher foliage surface, whereas during autumn, *Q. ilex* seedlings developed more strongly than *Q. pubescens* seedlings (greater height increment, higher number of leaves and higher foliage surface) benefitting from the autumnal return of rainfall events. These seasonal responses highlighted the phenological strategies employed by the two species to avoid summer drought: (i) the earlier and greater aerial development of *Q. pubescens* before summer drought is a typical growth strategy of Mediterranean winter deciduous species (Montserrat-Marti et al. 2009); (ii) the greater autumnal phenophases of the evergreen species *Q. ilex* is an adaptive strategy to cope with the dry period and take advantage of post-dry summer precipitation events (Valentini et al. 1992, Tognetti et al. 1998). The higher early performance of *Q. pubescens* is not explained by a higher seed mass for this species.

The greater ability of *Q. ilex* seedlings than *Q. pubescens* to react to the first autumnal rainfall events might be due to *Q. pubescens* being more sensitive to severe drought at the end of summer, as shown in other study on an adjacent area (Prévosto et al. 2011a). Furthermore, a previous study conducted during drier years (Prévosto et al., 2011b) found a much higher mortality rate in *Q pubescens* than *Q. ilex* seedlings, although our particularly wet climatic conditions did not cause significant mortality in either species. Our original survival results underlined the importance of considering specific wet years as particularly favourable when studying seedling regeneration under a Mediterranean climate (Gomez-Aparicio et al. 2005a). Finally, the phenological

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strategy of Q. *ilex* may prove a decisive advantage given the seasonal climatic changes predicted for the Mediterranean region. Spring phenological events are likely to be more affected by climatic conditions than during autumn and they will probably undergo the greatest alterations of climate (Gordo & Sanz 2010). The lesser climate alterations at the end of the plant cycle (autumn) than at the beginning of the plant cycle (spring) may therefore benefit species that demonstrate greater autumnal phenological activity, as observed here for *Q. ilex*.

Species-specific canopy effect on budburst and growth flushes

In April, at the first harvesting date, bud development was less advanced under Dense canopy than under Medium and Open canopy, in agreement with a previous study on Mediterranean *Quercus* sp (Sanz-Pérez & Castro-Díez 2010). Spring plant budburst timing has been reported to be controlled by soil and air warming, which was found to be higher under Open canopy in early spring in our study (see fig. 2 for soil temperature) (Bronson et al. 2009). Wetter and warmer conditions under Open canopy cover during early spring may thus favour faster leaf expansion and maturation, which are developmental behaviours strongly vulnerable to inhibition by dry conditions (Gratani & Bonito 2009, Misson et al. 2011).

Q. pubescens produced growth flushes during three periods (spring, early summer, and autumn) under Open canopy but during only one period under Dense and Medium canopy (spring). This absence of autumnal growth under closer canopy covers could be due to light shortage, lower temperature and higher water stress (see Prévosto et al. 2011a for the water status of *Quercus* seedlings in a adjacent area). In contrast, *Q. ilex* increased its growth activity (mean number of flushes and percentage of saplings growing) in two periods (spring and autumn) independently of canopy cover. This contrasted phenological response to canopy cover between the two species highlights a more conservative strategy of *Q. ilex* in response to heterogeneous micro-habitats conditions. *Q. pubescens* also showed greater pre-summer new leaf formation and a greater pre-summer flush activity in light-abundant habitats. Under a relative absence of summer mortality (as found here), this higher leafing and flushing could be an adaptive advantage for colonizing relatively open microsites, producing larger and more competitive seedlings. However, during more drastic drought periods, a higher investment in leaf development under high irradiance just before summer may also compromise seedling survival due to higher water losses (Fotelli 2000) and greater vulnerability to leaf hydraulic conductance in response to desiccation (Scoffoni et al. 2008). This trade-off between exploiting the most favourable environmental conditions and

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avoiding summer drought may thus compromise the survival of *Q. pubescens* seedlings in light-abundant habitats during drier years.

Litter depth: a short respite to keep growing during early summer

Indirect positive or negative effects of litter depth on oak seedling development are highly dependent on the species' phenological strategy. 67% of *Q. pubescens* seedlings produced a second flush of growth during early summer, when litter depth had a positive effect on soil moisture, whereas 77% of *Q. ilex* seedlings produced second flushes during the post-summer rainy season, when litter depth has a negative impact on soil moisture. This asynchronous flush production may explain the slight positive influence of litter depth on the height of the second *Q. pubescens* flush occurring in early summer and the absence of litter depth effect on the growth performance of *Q. ilex* seedlings in deeper litter layers. A previous study in a mixed-oak forest of SW Portugal found similar positive effects of litter depth on the seedling performance of another winter deciduous oak species (Maltez-Mouro et al. 2009). Thus, during early summer, the protection of soil moisture provided by thicker litter layers seems to benefit *Q. pubescens* seedlings more due to their greater phenological reactivity to sporadic rainfall. However, given the particularly wet conditions prevailing in the year studied, long-term observations are warranted in order to assess this protective effect of litter depth under more severe dry conditions.

Concluding remarks

Our findings suggest that altered microclimatic conditions favouring different microhabitats may either attenuate or accelerate the impacts of the predicted warming and altered watering patterns on the phenology and development of Mediterranean species, as reported by Miranda et al. (2009) and Morin et al. (2010). The range and dynamics of the Mediterranean pine forest remain uncertain under future drought scenarios (IPCC 2007). Our study underlines the importance of studying local climatic changes in conjunction with forest microhabitats in order to anticipate changes in the dynamics and composition of forest communities in the northernmost Mediterranean region of Europe. A recent IUCN report also highlighted this need, mentioning that microclimate conditions in the forest understory are extremely valuable for building up resilience against future climate change (Regato 2008). In a context of global climate change, there is a need for further insight into the effects of local microhabitats on the phenology of regenerating species in order to implement appropriate forest management policies and improve forest resilience.

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Table 1. Plant variables used for growth and phenological analyses.

Growth parameters	Definition
Growth duration	Number of sampling dates where individual is growing
Bud development	Mean of seedling bud stage at each sowing point at the first sampling date (3rd April)
Mean number of individuals growing per sowing point	For a sowing point: number of individuals growing / number of seedlings alive
Number of flushes	Individual number of growth flushes of the leader shoot 2009
Height	Individual height increment of the leader shoot 2009
Number of leaves	Individual number of leaves of the leader shoot 2009
Foliar area	Individual foliar area of the leader shoot 2009
% of pre-summer foliar area developed at 23rd April	(Foliar area at 23rd April * 100) / Foliar area at 24th June
% final height developed before dry summer	(Height at 24th June * 100) / Height at 17th December
% final number of leaves developed before summer	(Number of leaves at 24th June * 100) / Number of leaves at 17th December
% final foliar area developed before summer	(Foliar area at 24th June * 100) / Foliar area at 17th December
% final number of flushes developed before summer	(Number of flushes at 24th June * 100) / Number of flushes at 17th December

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Table 2. Values (mean ± SE) for the two species (*Q. ilex* and *Q. pubescens*) for (a) Bud development stage at 3rd April; (b) % pre-summer foliar area developed at 23rd April; (c) Number of pre-summer flushes at 24th June; (d to g) % final pre-summer development for height, number of leaves, foliar area and number of flushes, respectively. Species were significantly different in all measured variables (Mann-Whitney U test, all $p<0.0001$ except c. $p<0.01$)

	Q. ilex	*Q. pubescens*
a. Bud developmental stage at 03rd April	2.32 ± 0.12 a	3.36 ± 0.16 b
b. % of pre-summer foliar area developped at 23th April	20.76 ± 2.34 a	42.73 ± 3.66 b
c. Number of pre-summer flushes at 24th June	1.07 ± 0.04 a	1.2 ± 0.04 b
d. % of final height developed before summer	74.8 ± 2.63 a	94.23 ± 1.42 b
e. % of final number of leaves developed before summer	75.28 ± 2.62 a	94.19 ± 1.58 b
f. % of final foliar area developed before summer	72.89 ± 5 a	93.58 ± 2.41 b
g. % of final number of flushes developed before summer	73.22 ± 2.62 a	92.24 ± 1.65 b

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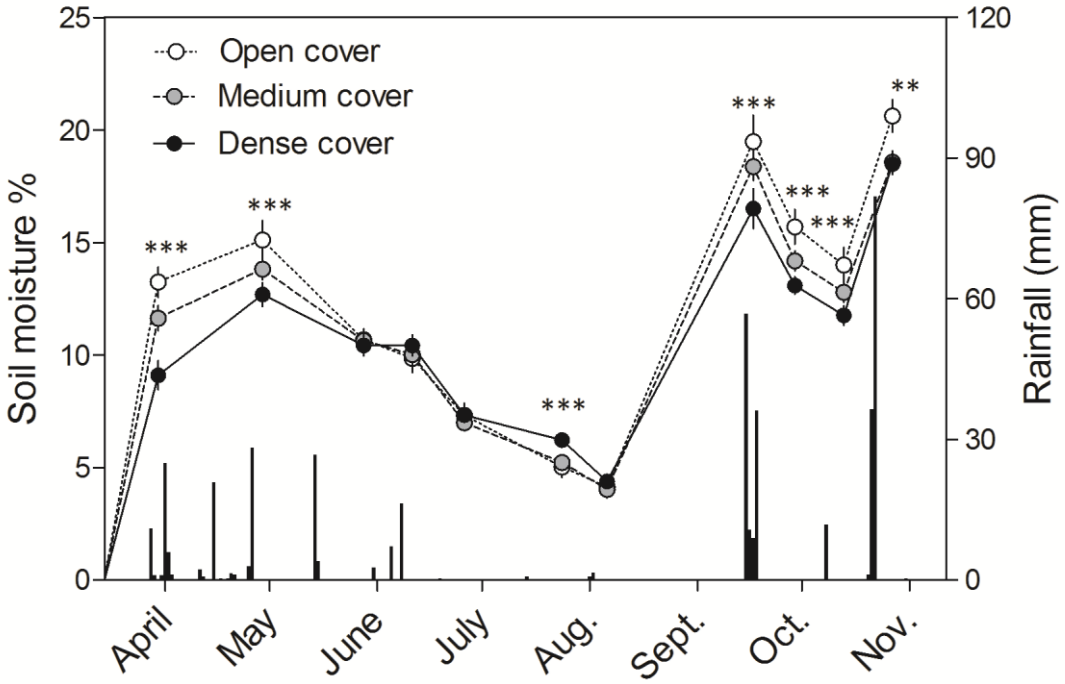

Fig. 1. Rainfall events and time-course of soil moisture over the 2009 growth period under Open (80-45%), Medium (45-20%) and Dense canopy cover (20-5%). Rainfall events are indicated by vertical black bars. Soil moisture values are means ± SEM. For a given date, statistically significant differences between canopy covers are indicated by asterisk(s) (* $p<0.05$; ** $p<0.01$; *** $p< 0.001$).

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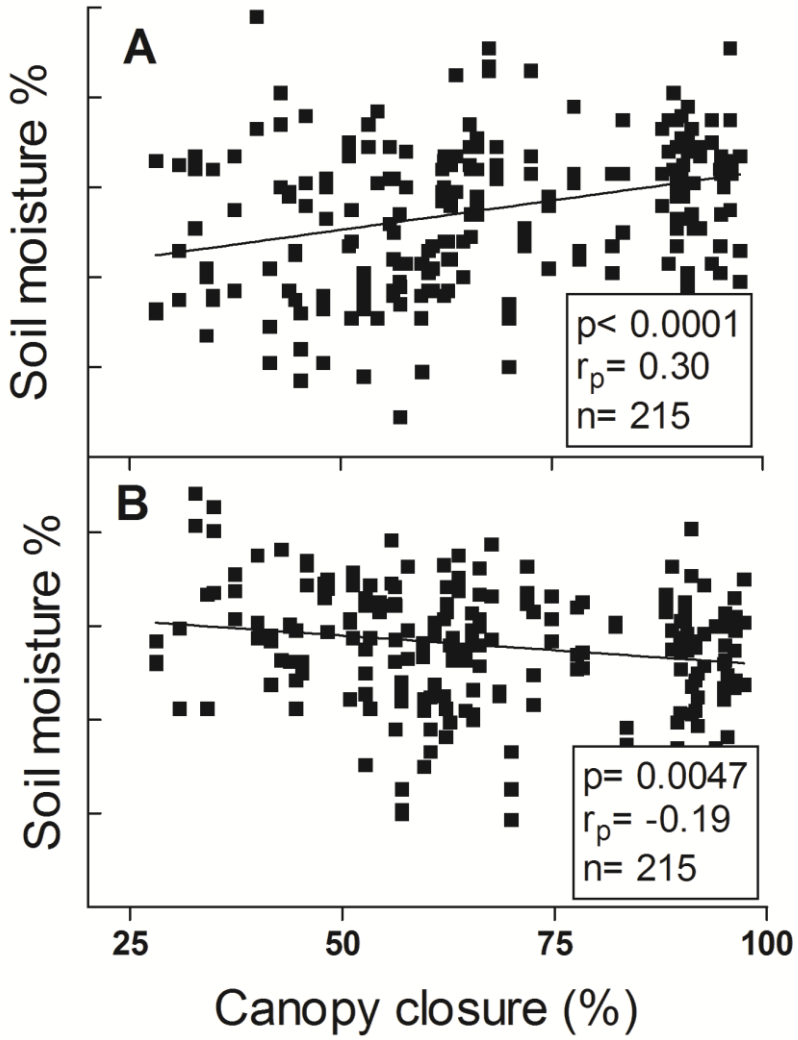

Fig. 2. Pearson's rank (rp) correlations between soil moisture and canopy cover (A) during dry summer (24th July) and between soil moisture and canopy cover closure (B) during a wet autumn (27th October).

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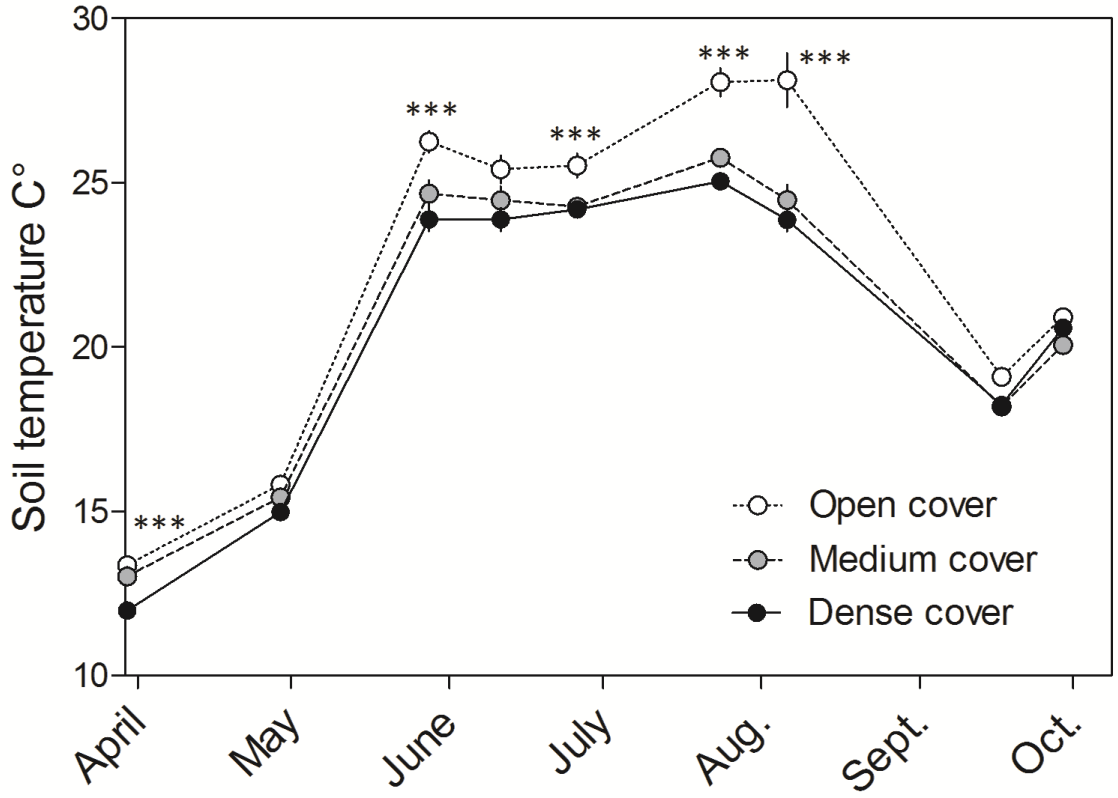

Fig. 3. Soil temperature over the 2009 growth period under Open (80-45%), Medium (45-20%) and Dense canopy cover (20-5%). For a given date, statistically significant differences between canopy covers are indicated by asterisk(s) (*: $p<0.05$; **: $p<0.01$; ***: $p< 0.001$).

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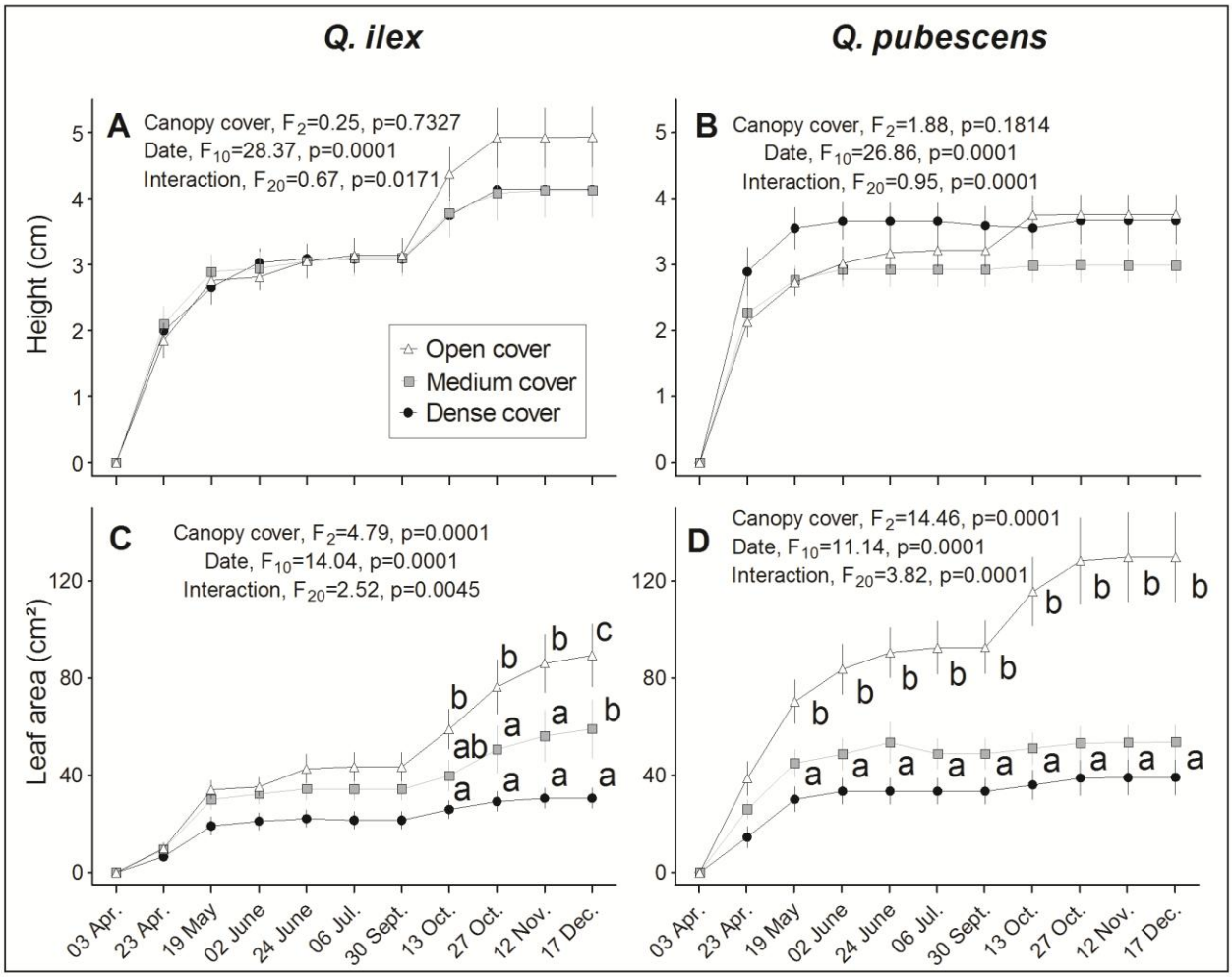

Fig. 4. Height (A, B) and Leaf area (C, D) of *Q. ilex* and *Q. pubescens* under Open (80-45%), Medium (45-20%) and Dense canopy cover (20-5%). Symbols are the mean of n plants sampled at each date, and error bars indicate SEM (two-way RM-ANOVA, Bonferroni post hoc test). Total of seedlings sampled in A-C is n= 27, 43, 36 and in B-D is n= 34, 49, 39 under Dense, Medium and Open canopy cover, respectively. When ANOVA was significant, different letters indicate statistical differences between canopy cover classes at each date (Bonferroni post hoc test).

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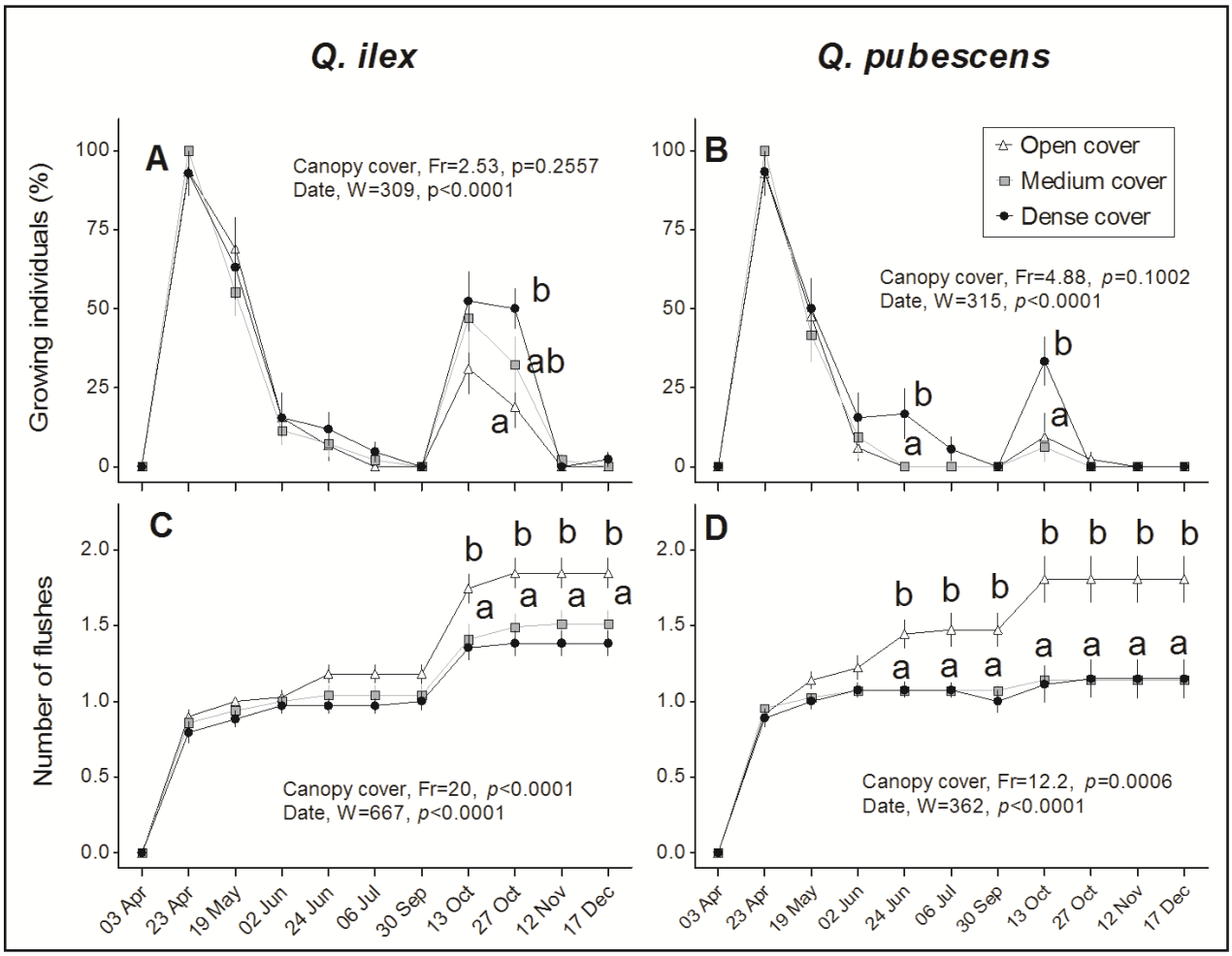

2 **Fig. 5.** Growing individuals (A, B) (%) and mean number of flushes (C, D) of *Q. ilex* and *Q. pubescens* under
3 Open (80-45%), Medium (45-20%) and Dense canopy cover (20-5%). Symbol are the mean of n plants sampled
4 at each date, and error bars indicate SEM (Friedman's non-parametric test, Dunn's post test). A: n=14, 16, 15; B:
5 n=15, 16, 14; C: n= 27, 43, 36; D: n= 34, 49, 39, in Dense, Medium and Open canopy cover, respectively. In A
6 and B, n corresponds to the percentage of growing individuals by sowing point. Date effect was tested by a
7 Kruskal-Wallis test followed by Dunn's post test. Letters indicate statistical differences (Kruskal-Wallis, P<0.05,
8 Dunn's post-test) between canopy closure classes at each date (Medium cover and Dense cover share the same
9 letters except in A).

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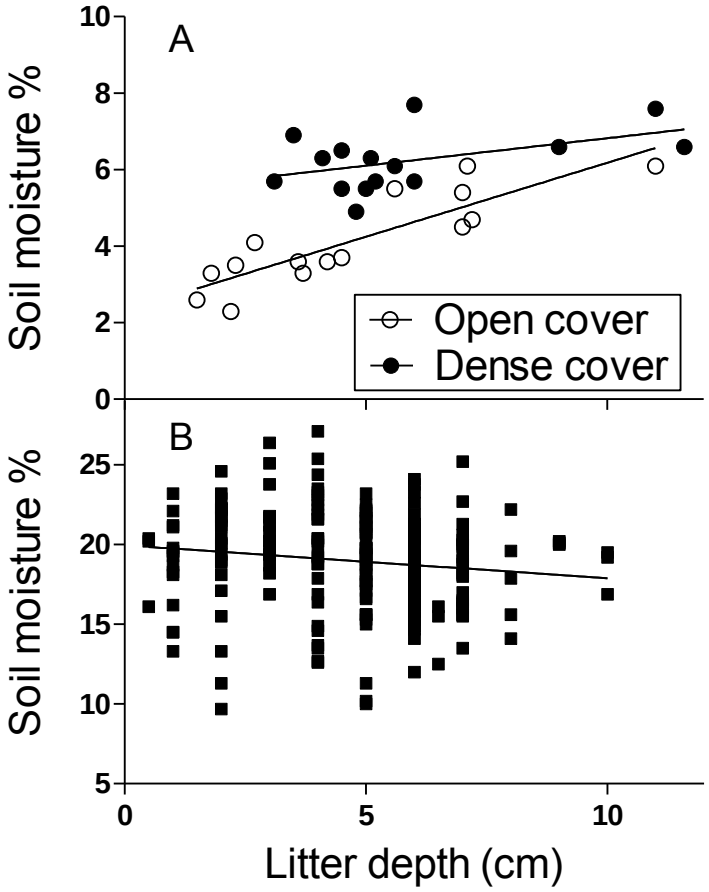

Fig. 6. Variation in soil moisture vs litter depth. (A) during dry summer (July), Pearson ranks (rp) correlations between soil moisture and litter depth under Dense canopy cover (dark circles) and Open canopy cover (Open circles), data points are means of three replicate measures sampled at the same sowing point, on 24th July 2009, regression lines are indicated for Dense canopy cover (n=15, slope=0.14±0.07, R^2=0.22, p>0.05) and Open canopy cover (n=15, slope = 0.37±0.06, R^2= 0.75, p<0.001); (B) during wet autumn (27th October), spearman rank (rs) correlations between soil moisture and litter depth